Cardiotoxicity of Tyrosine Kinase Inhibitors in Renal Cell Carcinoma.

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Introduction

Renal cell carcinoma (RCC) is the 6th most common cancer in the U.S., and nearly half of these patients develop cardiovascular (CV) side-effects from the gold-standard therapy—tyrosine kinase inhibitors (TKIs)[1-3]. Most patients with cancer now die from CV disease rather than from cancer itself. Hence it is imperative to explore the mechanisms by which these life-saving drugs induce heart failure (HF) in order to develop strategies to mitigate these effects and sustain cancer treatment for as long as possible.

Pazopanib is a TKI that inhibits vascular endothelial growth factor receptors (VEGFRs) and it is approved by the FDA for the treatment of various cancers including RCC. The clinical use of pazopanib and other TKIs is strongly limited by their association with serious CV side effects. Although little is known about the mechanism of TKI-induced cardiotoxicity, this drug causes serious CV effects in 50% of treated patients, including HTN, HF and myocardial ischemia[1-3]. We are currently using the murine model to study the effects of pazopanib *in-vivo*.

We have created a structural heart disease model in mice which display early signs of HF similar to humans. These mice (βIIS-cKO mice) lack cardiac βII-spectrin, a cytoskeletal protein that is important for the structural integrity of the cell. Our previous work has shown that these mice have arrhythmias, spontaneous Ca^{2+} release and abnormal expression/localization of cardiac membrane proteins[4]. Wild type (WT) mice with induced HF show downregulation of βII-spectrin accompanied by breakdown peptides which may serve as early blood and urine markers for HF.

Methods

Mice
8 Week old black male WT mice were orally dosed with 30 mg/kg of pazopanib twice daily for 42 days. Transgenic mice (βIIS-cKO mice) which lack cardiac βII-spectrin were observed at baseline.
Mouse Measurements
Organ weights and tibia length from mice were measured immediately after removal at the conclusion of the experiment.
Echocardiography (Echo)
The Small Animal Imaging Core's Vevo2100 echo machine was used to obtain images once per week.
Surface Electrocardiograms (ECG)
Surface ECG's were obtained once per week.
Immunoblots
Mouse whole heart lysates were electrophoresed and tested for several antibodies of interest.
Blood Pressure
The CODA system was used to gather non-invasive blood pressure readings once per week.
Electrophysiology
Ventricular cardiomyocytes were isolated from mice and used for electrophysiology testing *in vitro*.

Results

Mean arterial blood pressure over course of treatment

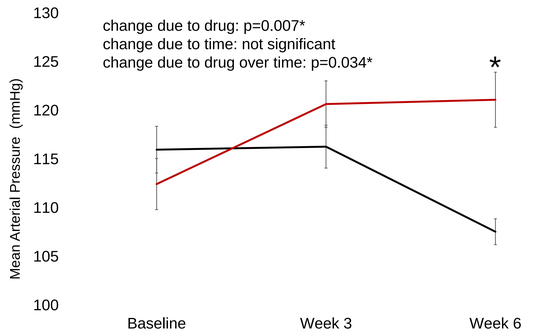
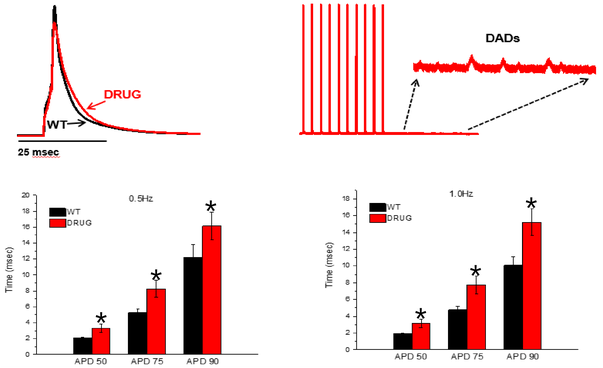


Figure 1. Mean arterial pressure over time of control group (black) compared to mice treated with pazopanib (red). A two-way ANOVA showed a significant increase ($p<0.05$) in mean arterial pressure due to drug treatment but not due to time (n=7). * *indicates statistical significance. Error bars represent ± standard error of mean.*

Electrophysiology results at conclusion of treatment



Hz/TREATMENT	APD50 +/- SE	APD75 +/- SE	APD90 +/- SE
0.5 WT	2.08 +/- 0.13	5.29 +/- 0.63	12.20 +/- 2.22
0.5 DRUG	3.33 +/- 0.60	8.26 +/- 1.32	16.14 +/- 2.12
1.0 WT	1.92 +/- 0.15	4.77 +/- 0.58	10.11 +/- 1.31
1.0 DRUG	3.13 +/- 0.58	7.74 +/- 1.27	15.20 +/- 1.96

Figure 2. Pazopanib treatment causes precursors to arrhythmias. Average action potential duration (APD) 50, 75 and 90 of myocytes from treated mice (red) was significantly elevated compared to control myocytes (black). 2/6 myocytes from treated mice developed delayed afterdepolarizations (DADs) while these were not observed in 5 control myocytes (n=2).

Protein expression at conclusion of treatment

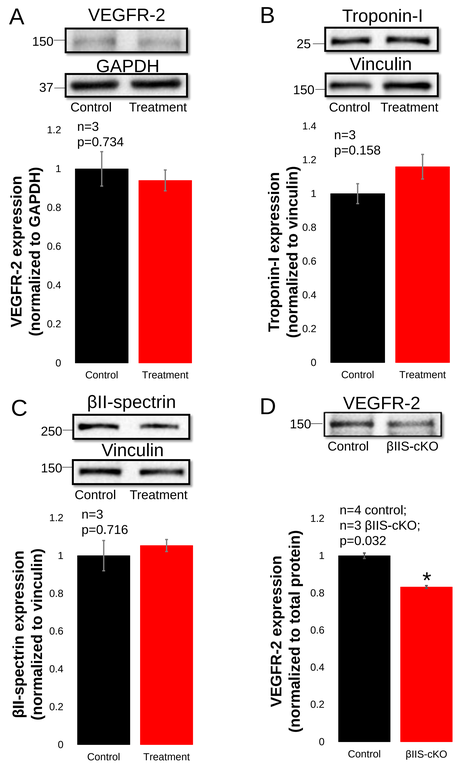


Figure 3. Immunoblots from WT whole heart tissue at the conclusion of pazopanib treatment (A-C) and from βIIS-cKO whole heart tissue at baseline (D). There were no significant changes in protein expression levels of βII-spectrin, VEGFR-2, or troponin-I (a biomarker for necrosis and apoptosis) (**A-C**). βIIS-cKO mice have significantly decreased expression of VEGFR-2 at baseline compared to control mice (**D**).

Echo, ECG and mouse measurements over course of treatment

Echoes did not show structural changes in the hearts of mice treated with pazopanib (n=7). ECGs did not show visible arrhythmias in treated mice (n=7). There were no significant changes in mouse measurements (weight, heart weight, kidney weight, brain weight and tibia length) due to treatment (n=7).

Discussion

These results in mice resemble the hypertensive CV effects of TKIs seen in humans and warrant further investigation. We plan to repeat this experiment using various susceptible mouse populations (**Fig. 4**) in order to observe advanced HF phenotypes. Transverse aortic constriction (TAC) will be used to induce HF. TAC mice and βIIS-cKO mice will be treated with pazopanib under the same dosing conditions used in this experiment. A portion of these mice will be treated with a renin-angiotensin-aldosterone antagonist (RAAA). Mass spectrometry will be used to identify βII-spectrin breakdown peptides in blood and urine from mice treated with pazopanib. This experimental design will allow us to:

- Unravel the cardiotoxic molecular mechanism of TKIs within the cell
- Assess the role of the renin-angiotensin-aldosterone (RAA) system in sustained HTN
- Observe the downstream effects of TKI treatment on βII-spectrin
- Test a potential new biomarker for early signs of TKI-induced cardiotoxicity

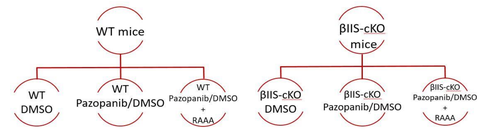


Figure 4. Experimental treatment design. Each WT group will have a companion group that undergoes TAC for a total of six WT groups. Each TAC animal will be paired with a sham-operated animal for comparison.

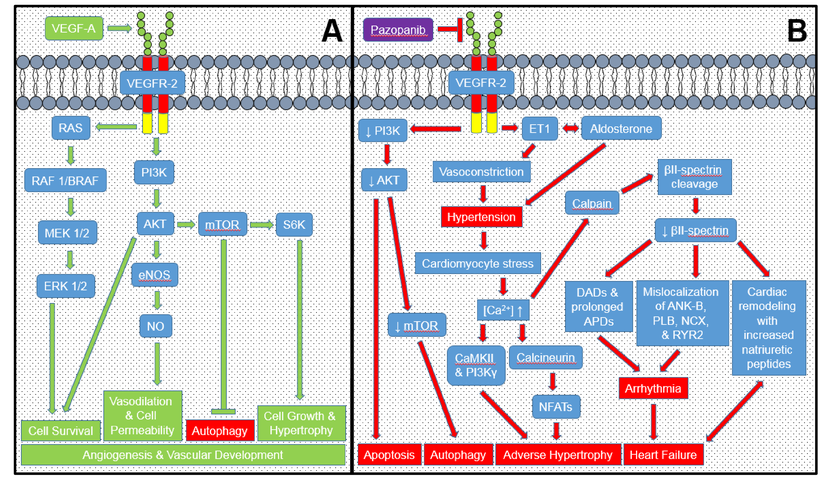


Figure 5. The cardiotoxicity of pazopanib and other TKIs may be linked to βII-spectrin and the RAA system. A shows the normal role of the VEGF signaling pathway in angiogenesis and vascular development (green). **B** shows the mechanism for pazopanib-induced disruption of angiogenesis and cardiac homeostasis (red). *mTOR, mechanistic target of rapamycin; CaMKII,* Ca^{2+}*/calmodulin-dependent protein kinase 2; PLB, phospholamban; RYR2, ryanodine receptor 2; NCX,* Na^{+}/Ca^{2+} *exchanger.*

References

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